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Murine and Humanized Constructs of Monoclonal Antibody M195 (Anti-CD33) for the Therapy of Acute Myelogenous Leukemia

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Long-term survival rates of patients with acute myelogenous leukemia treated with intensive chemotherapy are 15–20%, despite efforts to develop new treatment strategies. Murine M195 (^{131}I-M195), an anti-CD33, immunoglobulin (Ig) G2a monoclonal antibody has reactivity restricted to early myeloid cells and myeloid leukemic blasts but not hematopoietic progenitors. Previous trials in patients with relapsed or refractory myeloid leukemia showed that ^{131}I-M195 rapidly targeted to the bone marrow and internalized into target cells.

This article describes a therapeutic dose escalation study in which 24 patients received from 50 mCi/m^2 to 210 mCi/m^2 of ^{131}I-M195 in divided doses. Cytoreduction of peripheral cell counts and bone marrow blasts occurred without nonhematopoietic toxicity. Doses of ^{131}I-M195 greater than 135 mCi/m^2 were associated with marrow cytoreduction sufficient to necessitate bone marrow transplant. However, 37% of the patients developed human anti-mouse antibody, preventing retreatment.

To decrease immunogenicity and improve effector function, chimeric IgG1 and IgG3, and complementarity-determining region-grafted, humanized IgG1 and IgG3 versions of mouse M195 were developed by genetic engineering techniques. The new versions maintained specificity and biologic function, and they were superior to the mouse M195 in their ability to perform antibody-dependent cellular cytotoxicity against leukemia cells. Humanized M195, but not chimeric M195, showed a 4–8.6 times higher avidity than its mouse counterpart. Because effector function of IgG depends to a large extent on Fc clustering, a homodimeric HuG1 also was developed. Homodimeric HuG1 showed an ability to cause additional dramatic improvements in effector functions, as well as an ability to internalize and retain radioisotope in target leukemia cells. Monomeric and dimeric forms of humanized M195 may be advantageous in the therapy of acute myelogenous leukemia. *Cancer* 1994; 73:1049–56.

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Mouse monoclonal IgG2a antibody M195 binds to CD33, a 67-kilodalton (KD) glycoprotein. and is rapidly internalized into target cells.[1-3] It is specifically reactive with acute myelogenous leukemia (AML) cells and early myeloid progenitors, but not hematopoietic stem cells.[1,2] This sparing of normal stem cells, as well as its rapid accessibility to malignant cells in the blood, makes it an ideal candidate for leukemia therapy. Because M195 kills target cells with guinea pig and rabbit complement,[1] M195 has been used for ex vivo bone marrow purging of AML.[4]

Although M195 is not intrinsically cytotoxic in vitro, an initial pilot trial of 10 patients with myeloid leukemias showed rapid and specific bone marrow targeting and efficient internalization of ^{131}I-M195 into blasts.[5] Using an optimal biologic dose of antibody for radioimmunotherapy from this original trial, a dose escalation trial of ^{131}I-M195 for relapsed or refractory

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myeloid leukemias was conducted and the results are described in this report. Safe, leukemic cytoreduction was seen with ^{131}I-M195.

In the hope of improving effector function and to avoid neutralizing human anti-mouse antibodies (HAMA) responses, genetic engineering techniques have allowed for the production of chimeric IgG1 and IgG3 (ChG1, ChG3), combining mouse variable regions and human constant regions; and more fully humanized, "CDR-grafted" IgG1 and IgG3 (HuG1, HuG3), so that only the original mouse antigen binding sites are retained.[6] Avoidance of HAMA may allow for longer half-lives in vivo and the ability for repeated treatments.[7] In addition, humanized monoclonal antibodies (MoAb) may obviate the need for toxins or radiolabels. Several humanized MoAb, including CAMPATH-1H,[8] anti-Tac-H,[9] and humanized versions of two murine MoAb against herpes simplex virus gB and gD glycoproteins,[10] offer selective advantages over their murine counterparts.

Hence, this report also describes new humanized constructs of M195. These humanized versions showed superiority over their murine parent MoAb in immune effector function and higher avidity than the original MoAb.[11] To further attempt to improve on biologic and immunologic effector functions of HuG1, a mutation in the heavy chain gene allowed disulfide bonds to form a homodimeric HuG1 (HdIgG). HdIgG showed dramatic improvements over the monomer,[12] in part, similar to that seen previously for a chimeric IgG dimer.[13] The potential use of humanized M195 constructs in the therapy of AML is discussed.

Materials and Methods

Cells and Cell Lines

Hematopoietic cell lines, including SKLy16, RAJI, HL60, and U937 cells, were obtained from the human tumor banks of the Cancer Immunology Laboratory at Sloan-Kettering Institute. Hematopoietic cell lines were grown in RPMI1640/5% newborn calf serum/10% serum-plus (Hazelton Biologics, Lenexa, KS). Heparinized peripheral blood samples were obtained from healthy volunteers and patients on the Leukemia Service at Memorial Hospital on IRB-approved protocols. Peripheral blood mononuclear cells (PBMC) were separated on Ficoll-Paque (Pharmacia, Piscataway, NJ) gradient centrifugation.

Monoclonal Antibodies

Monoclonal antibody M195 (anti-CD33) originated in Balb/c mice immunized with leukemia cells from a patient with AML, and was produced from hybridomas and purified as described previously.[1,2] Sp2/0 hybridoma cell lines secreting the chimeric and humanized M195 were grown in vitro, and the MoAb were purified on PA-Sepharose (Pharmacia, Piscataway, NJ) by affinity chromatography using sequential pH step elutions.[1] Purity was determined on sodium dodecyl sulfate (SDS)-polyacrylamide gels stained with Coomassie brilliant blue. Purification of clinical grade M195 was done from mouse ascites at Sloan-Kettering Institute essentially as described.[14] The product was tested for the presence of murine viruses, endotoxin, pyrogen, bacteria, fungal contamination, mycoplasma, and DNA. General safety testing was done on mice and guinea pigs. Human-mouse chimeric IgG, with human-derived constant regions for IgG1 and IgG3, and "CDR-grafted" humanized M195, retaining only the CDRs and other sterically important amino acids from the mouse IgG, were constructed as described.[6] The construction of homodimeric IgG (HdIgG) was recently described[12] by introducing a mutation at the $\gamma 1$ chain CH3 region gene to change a serine to a cysteine, allowing interchain disulfide bond formation at the COOH terminal of the IgG.

Radioiodination and Radioimmunoassay

For the clinical trial, 2 mg M195 was labeled with an appropriate amount of iodine-131 (New England Nuclear, Wilmington, DE) using chloramine-T and aseptic pyrogen-free technique.[15] In the initial M195 trial, optimal bone marrow targeting and minimal blood pooling occurred when less than 5 mg/M^2 antibody was infused. In addition, pharmacokinetics correlated with tumor burden.[5] Hence, in this trial, to maximize ^{131}I delivery to the marrow, M195 doses were adjusted for estimated leukemia burden: unlabeled M195 was added to bring the total antibody dose to 3 mg/M^2 plus an additional 0.1 mg/M^2 for every 10,000 peripheral white blood cells per microliter at the time of infusion.[15] This escalation was based on the known number of target sites per leukemia cell.[1] Doses for injection were greater than 98% free of ^{131}I and showed immunoreactivities of about 60% in one-step binding assay in antigen excess.[15]

Immunoreactivity of the new constructs was determined by incubating 3–5 ng of radiolabeled MoAb with an excess of antigen (5×10^6) HL60 cells for 1 hour at 4°C. Specific binding in these radiobinding assays was determined by subtracting the amount of radiolabeled MoAb bound in the presence of an excess of unlabeled MoAb as previously described.[1]

Trial Design

Details of the trial and its design have been published.[15] Patients with relapsed or refractory acute nonlymphocytic leukemia, blastic chronic myelogenous leukemia or myelodysplastic syndrome (MDS), or chronic myelomonocytic leukemia were eligible if their blasts expressed CD33 antigen in greater than 25% of cells by flow cytometry. All patients were not undergoing chemotherapy (except for hydroxyurea) for at least 3 weeks before entering this study. Hydroxyurea was permitted to control peripheral blood counts up to 2 days before beginning treatment. Entry criteria included serum creatinine and bilirubin less than 2 times normal values, no positive blood cultures for bacteria within the preceding 10 days and Karnofsky Performance Status (KPS $\geq$ 50%).

Seven ^{131}I dose levels were employed: 50, 70, 90, 105, 135, 160, and 210 mCi/M^2. A minimum of three patients were treated at each dose level. Because the possibility of irreversible marrow ablation became apparent at higher dose levels, patients were treated with 160 mCi/M^2 or greater only if they were eligible for allogeneic or autologous (prior stored remission marrow) transplantation.

^{131}I-M195 was administered by 20-minute intravenous infusion in 2–4 divided doses given at least 48 hours apart to allow for reexpression of the CD33 antigen on the surface of target cells between infusions. Physical examination, complete blood cell count, coagulation indices, and biochemical and electrolyte values were measured daily. Before the second or third antibody infusions, patients underwent whole-body anterior and posterior gamma camera imaging.[15]

Toxicity was assessed according to the common criteria established by the National Cancer Institute. This trial was conducted under IRB approval, with informed consent or assent obtained from all participants.

Flow Cytometry

Peripheral blood cells from selected patients were examined for expression of M195 antigen (CD33) and other myeloid cell surface markers before, 1 hour, and 24 hours after treatment with M195, using an EPIC Profile flow cytometer.[1] Goat anti-mouse Ig-fluorescein isothiocyanate conjugate was used without primary antibody to coat and detect M195 bound cells in vivo. Specificity studies of the humanized constructs were done by direct and indirect flow cytometry using goat anti-human Ig-fluoroscein isothiocyanate conjugate as described.[11]

Quantitation of Killing of Bone Marrow Blasts

Bone marrow biopsies taken before therapy and at least 7 days after completion of therapy were assessed for absolute mononuclear cell count per millimeter of marrow sample, using the technique of Blumenreich et al.[16] The values were multiplied by the percentage of blasts present on a simultaneously obtained marrow aspirate smear to calculate the absolute number of blasts present per millimeter of sample. Values before and after treatment were compared to determine the resulting change in absolute blast concentration.

Human Anti-Mouse Antibody

Plasma samples were tested for HAMA beginning 8 days after the last infusion and then every 2–3 weeks thereafter using an enzyme-linked immunosorbent assay against purified mouse M195 and human-mouse chimeric M195[6] essentially as described.[14]

Avidity Determinations

Scatchard analyses were done as previously described.[9] Comparison of antigen–antibody avidity between different M195 constructs was done in competition with ^{125}I-M195 and ^{125}I-HuG1. Increasing amounts of cold MoAb were incubated with 2×10^5 HL60 cells and 50 ng ^{125}I-MoAb in 200 μl RPMI for 1 hour at 0°C.[11] Cells were washed 3 times in RPMI and counted. To avoid nonspecific and Fc-receptor binding, competition assays were done in the presence of human serum. Samples were run in duplicate and the avidity of binding was measured by comparing the concentrations of unlabeled competitor where 50% reduction of binding was achieved as described.[9] Experiments were repeated 2–3 times each, and arithmetic means of the data are presented.

Internalization Studies

Internalization of the HuG1 or HdIgG was measured from 0 to 48 hours by incubating 0.01 μg/ml to 2 μg/ml radiolabeled MoAb with 1–2 million HL60 cells/ml in RPMI 1640/2% human AB serum.[11,12] Cells then were washed twice in RPMI, and the surface-bound M195 was stripped with 1 ml 50 mM glycine/150 mM NaCl, pH 2.8 at 24°C for 10 minutes. The amount (ng) of MoAb per million cells remaining after the acid wash (i.e., internalized), or in the supernatant (i.e., cell surface) was determined.

Complement-Mediated Cytotoxicity

Twenty-five microliters of HL60 cells (5×10^6 cells/ml) or fresh leukemia samples were incubated with 25 μl diluted rabbit or human complement and 25 μl serial dilutions of MoAb at 37°C for 60 minutes.[11] Monoclonal antibody M31 (IgM anti-CD15) was used as a positive control. Live and dead cells were enumerated using trypan blue or propidium iodide exclusion.

Rabbit serum was purchased from PelFreeze (Rogers, AK); human sera were obtained from volunteers. All complement sources were stored at −70°C until use and were not reused. Complement was used at the maximum concentrations not showing nonspecific lysis of the target cells, usually from 1:6 to 1:10 final dilution.

Antibody-Dependent Cellular Cytotoxicity

Chromium release assays to determine if chimeric, humanized, or homodimeric M195 MoAb were capable of mediating antibody-dependent cellular cytotoxicity (ADCC) were conducted as described previously.[1] Peripheral blood mononuclear cells from human volunteers were used as effector cells, and HL60 cells were used as positive targets. Assays were conducted at 37°C for 5 hours and harvested using a Skatron press, and released ^{51}Cr was counted in a Packard gamma counter. Detergent lysed cells were used as a 100% control. Effector cell only and MoAb only treated target cells were used as negative controls. CD33-negative RAJI cells and a control HuG1 MoAb (Fd 79)[10] were used as controls. Samples were done in quadruplicate and the mean was presented. Experiments were repeated 3–5 times. Standard deviations were always less than 10% of the mean value.

Results

Patient Studies

As described previously,[15] the 24 patients on study included 16 with refractory or relapsed AML, 5 with blastic MDS, 2 with chemotherapy-related secondary AML, and 1 with myeloblastic CML. Patients were heavily pretreated with chemotherapy, and 7 of these 24 patients had relapsed after allogeneic (n = 6) or autologous (n = 7) bone marrow transplantation. Patient median age was 40 years.

Marked uptake of ^{131}I-M195 into all areas of bone marrow as well as variable blood pooling could be seen by whole-body imaging at 48 or 72 hours. Flow cytometric studies using peripheral blood from 11 patients with adequate numbers of circulating leukemia cells were done to demonstrate the presence of surface bound M195 after infusion. Using direct immunofluorescence with MY9 (CD33), mean peak channel changes showed that 29% (range, 18–47%) of available CD33 sites were occupied at 1 hour, with concomitant binding of FITC-goat anti-mouse Ig (GAM) on a mean of 67% (range, 42–89%) of gated cells.

Table 1. Cytoreduction of Leukemia Cells in Blood and Bone Marrow After ^{131}I-M195 Therapy

	Level	Dose*	Decreased blood counts	Bone marrow depletion†
I	3 Patients	50	2/3	2/3
II	4 Patients	70	4/4	2/3
	1 Retreated			
III	3 Patients	90	3/3	2/2
	1 Retreated			
IV	3 Patients	105	3/3	3/3
V	2 Patients	135	2/2	2/2
VI	6 Patients	160	6/6	6/6
VII	3 Patients	210	3/3	3/3

* ^{131}I-M195 was administered by 20-minute intravenous infusion in two to four divided doses.

† Biopsies were taken prior to therapy and were assessed for absolute mononuclear cell count per millimeter of marrow at least 7 days after completion of therapy.

The cytoreductive effect of ^{131}I-M195 on peripheral blood and bone marrow is seen in Table 1. Modest to significant drops in peripheral white and leukemia cell counts were seen in all but one patient (96%). Although the duration of nadir was variable (6–43 days) and not directly dose dependent at lower dose levels (up to and including 105 mCi/m^2), pancytopenia was profound and long in duration (12 days or greater) at dose levels greater than 105 mCi/m^2. These included eight patients who underwent bone marrow transplantation, who previously had been ineligible for BMT due to refractory or relapsed leukemia. Bone marrow examination showed a direct correlation between ^{131}I dose level and mean blasts killed, with 17 of 19 (89%) of patients tested demonstrating decreases in the absolute number of blasts per millimeter of bone marrow biopsy.

Despite large cell kills, up to 1 kg (10^{12} cells), no laboratory evidence of tumor lysis or disseminated intravascular coagulation (DIC) was demonstrated. More than 99% of blasts were killed in some patients. No complete responses were seen at nontransplant dose levels. Clinical parameters were improved, however. A patient with acute promyelocytic leukemia who was in fulminant DIC before treatment showed clinical and laboratory resolution of DIC after treatment. Another patient who had transformed into AML from MDS, and had been refractory to chemotherapy, returned to her baseline MDS for a duration of 3 months.

The presence of HAMA in sera was examined. Thirty-seven percent of the patients developed a positive titer. In two patients that showed positive titers, retreatment with ^{131}I-M195 was unsuccessful, with poor bone marrow targeting and absence of cytoreduction. The only acute toxicities included bone pain and low-grade fever in each of four patients. One patient who developed hyperbilirubinemia with venoocclusive disease during her initial transplant developed hyperbilirubinemia on this study. Hematologic toxicity was difficult to evaluate because of baseline pancytopenia seen in these patients.

Humanized Antibodies

Specificity, Immunoreactivity, and Avidity of Humanized Antibodies

Because of the encouraging results of these clinical trials, and to reduce the likelihood of HAMA formation, humanized constructs were produced[6] and their biologic and immunologic characteristics were examined.[11,12] Chimeric and humanized IgG1 and IgG3, as well as homodimeric humanized IgG1, were analyzed for their specificity relative to the parental mouse M195. When tested against a panel of CD33$^+$ and CD33$^-$ cell lines by radioimmunoassay, all constructs maintained specificity. Specificity of the chimeric and humanized M195 was tested against fresh hematopoietic samples from 47 patients using a direct fluorescein conjugate of HuG1-M195, and confirmed for myeloid leukemia cells.[11]

The ability of the genetically engineered monomeric and dimeric constructs to bind to CD33 expressing HL60 cells was determined by radiobinding assays by incubating 3–5 ng of MoAb in the presence of excess antigen. Total immunoreactivity of the four radiolabeled constructs was 50%, similar to that of the original M195. This value nearly doubled to 85% for the HdIgG.

Direct radioimmunoassay of the constructs showed that binding was both specific and saturable. Scatchard analysis has shown that although the chimeric M195 showed similar or slightly lower avidity than the mouse M195, to our surprise both HuG1 and HuG3 showed a higher avidity than the parent Ig.[6] Not unexpectedly, HdIgG showed similar avidity to HuG1-M195.[12]

Because radiolabeling of the MoAb may cause damage during the radioiodination of the Ig, avidities were compared between different constructs and ^{125}I-M195 or ^{125}I-HuG1 using competition radiobinding assays.[11] The higher avidities of the humanized M195 determined by Scatchard analysis were confirmed by these assays. HuG1 and HuG3 showed an 8.6- and 4-fold higher avidity, respectively, than the mouse M195. The avidities of the ChG1 and ChG3 were confirmed by competition assays to be similar to that of the mouse.

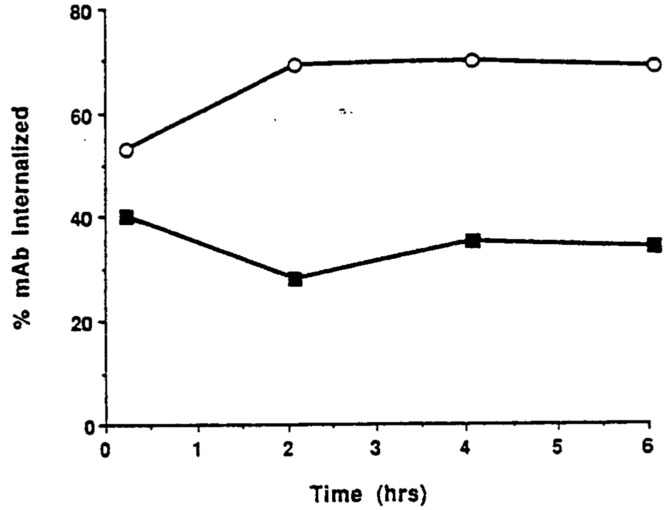

Figure 1. Percentage of HuG1 (■-■) and HdIgG (O-O) internalized over 6 hours at 37°C. Radiolabeled monoclonal antibody at 1 μg/ml was incubated with 2 × 10^6 HL60 cells in a final volume of 200 μl, and internalization was measured as described in the text. Each time point was done in triplicate and SD less than 10%.

Internalization

It was known from previous studies that M195 rapidly internalized into target cells, which was important for the therapeutic delivery of radioisotope into cells.[5] Both radiolabeled HuG1 and HuG3 became associated over time in an acid-resistant compartment (i.e., not on the cell surface) at 37°C. By 4 hours, 30% of the radiolabel was internalized into the cell. In contrast, HdIgG internalized much more rapidly than HuG1, with 70% of the radiolabeled MoAb being retained inside the cell by 4 hours (Fig. 1). Experiments performed at 0°C did not show internalization of any of the constructs. An extended analysis over 48 hours showed persistence of HdIgG within cells, demonstrating that dimerization extended the length of time that M195 could be retained inside the cell after binding to the surface.[12]

Effector Function

One of the main purposes of studying humanized MoAb is to determine whether they are more effective at cell killing by immune effector functions, i.e., complement-mediated cytotoxicity (CMC) or ADCC. All of the constructs showed HL60 leukemia cell killing using rabbit complement, but not human serum, as a source of complement.[11] For rabbit CMC, the murine and new monomeric constructs yielded 50% cell lysis at a MoAb

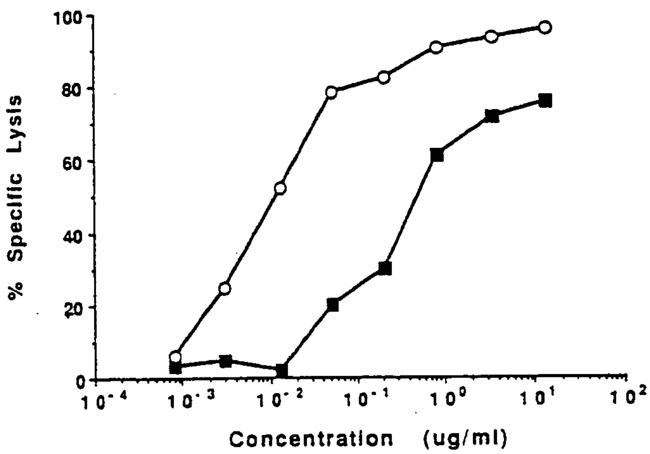

Figure 2. Complement-mediated cytotoxicity of HuG1 (■-■) and HdIgG (O-O). Dilutions of monoclonal antibodies were added to 10^5 HL60 cells with rabbit complement at final dilutions of 1:6 at 37°C for 1 hour. Cell kill was determined by trypan blue exclusion.

concentration of 0.1–0.5 μg/ml. All constructs showed a prozone effect, with decreases in activity at higher concentrations. HdIgG was at least 100-fold more potent on a molar basis at rabbit complement-mediated cytotoxicity than was HuG1-M195 (Fig. 2). Although no cytotoxicity for HuG1 was seen below a concentration of 0.01 μg/ml, HdIgG showed a linear increase in cell lysis starting at a concentration of 0.001 μg/ml.[12]

Unlike the mouse version, both chimeric and humanized M195 possessed the new capability of ADCC.[11] Cell killing in the range of 20–30% for ChG1 and ChG3, and 10–20% for HuG1 and HuG3, was seen in 5-hour chromium release assays at high E:T ratios (above 50:1) and MoAb concentrations 1 μg/ml and above. Killing by ADCC was linearly correlated with increasing E:T ratios (Fig. 3). Monoclonal antibody levels greater than 10 μg/ml did not demonstrate any improved level of killing, and in some cases, reduced levels of killing at higher antibody concentrations were seen. No killing was seen with a control HuG1 MoAb (Fd79)[10] and HL60 cells, or with HuG1-M195 and CD33$^-$ RAJI cell lines. Similar to CMC, HdIgG was also 100-fold more potent on a molar basis at ADCC than HuG1.[12] At each E:T ratio, HdIgG was 2- to 5-fold more effective over a 50-fold concentration range.

Discussion

This report summarizes clinical work with ^{131}I-M195 and the development of newly designed humanized versions with their improved cytotoxicity and biologic functions. This was the first trial of a monoclonal antibody with significant single agent activity against AML in vivo.[15] This therapeutic trial of radiolabeled M195 in patients with relapsed and refractory myeloid leukemia has shown M195 to be an effective agent for cytoreduction without nonhematologic toxicity. Peripheral blood and bone marrow involvement was decreased in more than 90% of patients. M195 is capable of destroying up to nearly 1 kg of leukemia in patients without tumor lysis. The amount of blasts killed (up to 2.5 logs) compared favorably with that reported for anthracycline/cytosine-arabinoside therapy.[16] Despite dose escalation from 50 to 210 mCi/m^2, the maximum tolerated dose of ^{131}I-M195 was not reached, based on dose-limiting nonhematologic toxicity. Hematologic toxicity appeared to be based not only on the radiation dose level, but also on the previous treatment affecting the patient's bone marrow reserve.

The amount of cell kill from ^{131}I beta-emission is highly dependent on the concentration of CD33 target, in addition to the "field of kill effect" due to the range of emission of 50 cell diameters for beta emitters. This predicts that ^{131}I-M195 is useful for marrow ablation for patients in relapse. Doses greater than 135 mCi/m^2 ^{131}I caused prolonged pancytopenia of longer than 14 days. In eight patients who were previously not candidates for bone marrow transplantation, leukemia blast reduction with M195 was sufficient to proceed to transplantation.

It was previously shown that M195 is capable of rapidly targeting to areas of bone marrow with leukemic involvement while sparing normal tissues.[7] In this study, successful bone marrow imaging was demonstrated as late as 72 hours and at least 168 hours into a course of MoAb with relatively low doses (3 mg/m^2) of MoAb administration. Cellular targeting was confirmed

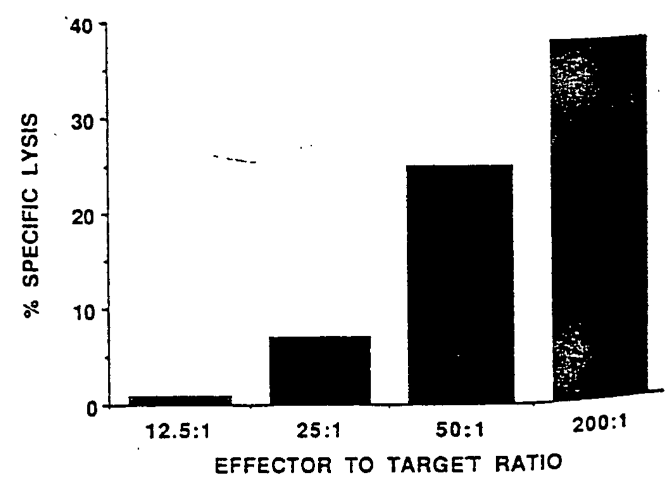

Figure 3. Antibody-dependent cellular cytotoxicity of HuG1 as a function of E:T ratio. Target HL60 cells were incubated at 37°C with monoclonal antibody at 1 μg/ml and peripheral blood mononuclear cells as effectors. ^{51}Cr-release was measured 5 hours later as described in the text.

by flow cytometry, and this dose was more than sufficient to target all cells with minimal excess. The combination of low nontarget doses and high specific activity of labeled antibody make it an exceptionally effective agent for massive cell kill.

Retreatment in two patients with HAMA resulted in rapid clearance of antibody and reduced effectiveness of the mouse MoAb.[15] To avoid neutralizing HAMA responses, and to take advantage of natural immune effector functions, obviating the need for radioisotopes and immunotoxins, humanized versions of M195 were constructed. These newly genetically engineered chimeric and humanized M195 constructs show improvements over the murine MoAb in both avidity and recruitment of host effector cells.

To our surprise, the humanized, but not chimeric versions, show a markedly higher avidity than that of the original mouse MoAb.[11] Competition experiments using either ^{125}I-HuG1 or ^{125}I-M195 corroborated the suggested higher avidities of HuG1 and HuG3, as calculated from the Scatchard plots.[6] These results are probably more accurate than the Scatchard plots because radioiodination may affect binding characteristics. Other humanized MoAb, including anti–TAC-H[9] and anti-Herpes MoAb[10] showed slightly lower, but not improved, avidities compared with the original parental murine MoAb. Comparisons of the variable region sequences of M195[6] showed a loss of a carbohydrate binding site in the variable region from the CDR-grafted M195 MoAb. This change in the carbohydrate region may be responsible for the dramatic difference in avidities of HuG1 and HuG3. This hypothesis is being investigated.

The ability of M195 to internalize and carry radioactivity into target cells is crucial to the in vivo therapeutic effects seen in this study, or for the use of toxins to purge bone marrows of AML cells in vitro.[17] The ability of M195 to persist inside cells as shown here by flow cytometry is in contrast to studies of ^{131}I-P67, which was poorly retained by cells, because of rapid endocytosis and expulsion of degraded metabolites.[18] HdIgG was more than 2 times more efficient than any monomeric M195 at staying inside the cell, in light of a similar binding avidity. This more efficient internalization held true over 48 hours. Thus, radiation doses to target cells would be twice as high for the same added dose and same toxicity. Although others have found that cross-linked IgG bound to Fc receptors more avidly than monomeric Ig,[19–21] our system is unique in that binding and internalization occur through specific Fab binding to CD33 receptors.

Patients with AML who received infusion of autologous marrow treated with anti-MY9 and rabbit complement after myeloablative chemotherapy showed trilineage engraftment and remission.[22] Although all of the monomeric versions performed CMC with rabbit complement,[11] HdIgG was at least 100-fold more potent at cell killing when compared with HuG1.[12] This was most likely due to the proximity of Fc receptors, because binding and activation of C1q requires the formation of doublet IgG on the cell surface.[23] The ability of HdIgG to allow spatial arrangements to occur is consistent with the mechanism of a cluster of IgGs needed for the attachment of C1q,[24–26] or for the critical spacing of epitopes on the cell surface necessary for CMC to occur.[27] The additional evidence that bispecific antibodies that are capable of binding to two antigens,[28] and a chimeric antibody with dual Fc regions[29] were superior to monomeric MoAb in CMC, attests to the improvement seen with HdIgG.

Although HuG1[11] and HdIgG[12] were capable of fixing human complement, neither MoAb lysed HL60 cells in the presence of human complement. The ability of M195 to perform CMC using human complement was a function of CD33 antigen density. Effective killing of CD33$^+$ AL67 fibroblasts, but not HL60 cells with a 27-fold lower antigen density, was seen with human serum as a source of complement.[12]

One of the advantages of chimeric and humanized M195 is their ability to perform ADCC with human PBMC. This new effector function over the original murine MoAb has been reported for other chimeric[9,30] and humanized[9] MoAb. The much improved level of ADCC with HdIgG can be attributed to dual Fc receptors that would allow effectors and targets to be in close proximity. The spacing of IgG on the target cell surface is crucial for the enhanced susceptibility of K lymphocytes to bind and lyse antibody-coated red blood cells.[31]

The improved biologic properties and effector functions of humanized and homodimeric IgG1 M195 show promise for future in vivo therapies of AML. This clinical trial validates the use of radiolabeled mouse M195 for cytoreduction and as a preconditioning regimen at higher doses of radioactivity for allogeneic BMT in refractory leukemia. But it is our hope that radiolabels and immunotoxins will be replaced by the use of humanized monomeric or dimeric M195, alone or in combination with cytokines, for the treatment of myeloid leukemias. The most likely use of humanized versions of M195 will be for the elimination of minimal residual disease after chemotherapy, with the aim of preventing relapse and sustaining durable remissions in patients with myeloid leukemia.

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